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Effects of Short-Term Exposures to Sulfuric Acid and Ammonium Sulfate Aerosols Upon Bronchial Airway Function in the Donkey

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16. ABSTRACT

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Effects of short-term exposures to sulfuric acid and ammonium sulfate aerosols upon bronchial airway function in the donkey*

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introduction

Sulfuric acid (H_2SO_4) and ammonium sulfate ($(NH_4)_2SO_4$) are significant mass fractions of the 0.1 to 1.0 μm size accumulation mode of the ambient aerosol in many urban and rural areas,[1-5] primarily evolving from gaseous precursors through atmospheric reactions.[6] Increased concern about the health effects to the general population of these sulfur oxide aerosols developed largely due to the finding that the airborne concentration of water soluble sulfates correlated better with morbidity indices than did any other measured class of pollutant.[7]

In addition, sulfuric acid is used or produced in numerous industrial processes. Procedures which involve the rapid condensation of vapors from acid solutions and electrolytic processes using dilute acid solutions, for example, produce mists with particle sizes 1 μm or less. The air both in and around these plants is often contaminated with acid, providing the potential for occupational as well as community exposures.

A study was undertaken to assess and characterize the effects of short-term (one hour) inhalation exposure to submicron aerosols of ammonium sulfate and sulfuric acid upon certain aspects of bronchial airway physiology, using the donkey as an animal model. Indices of response were changes in: (1) respiratory mechanics, expressed in terms of pulmonary resistance and dynamic compliance, (2) the pattern of regional deposition of an inert test aerosol, and (3) the tracheobronchial mucociliary clearance rate of the inert aerosol. These physiological functions have been widely studied in both experimental animals and humans exposed to respiratory irritants.

methods

experimental animal

Four healthy, adult donkeys were used in this study. Work performed in this laboratory[8-11] has established the utility of the donkey as an experimental animal. Although the donkey has not been previously used for studies of respiratory mechanics, clearance changes consistent with and quantitatively comparable

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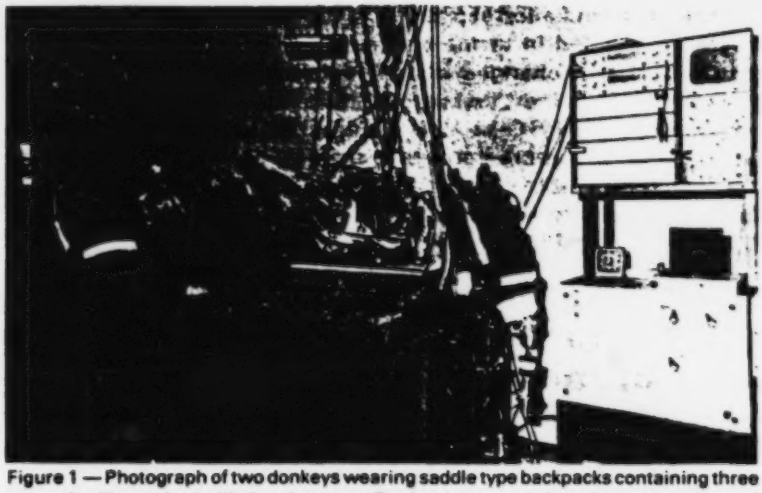

Figure 1 — Photograph of two donkeys wearing saddle type backpacks containing three pairs of collimated scintillation detectors. Donkey in center is breathing through nasal catheters connected to a screen-type pneumotacograph. Smaller catheters extend to balloons in nasopharynx and esophagus. Flow, volume and pressure tracings are displayed and recorded on apparatus at right.

to those seen in parallel human studies[12-14] have been observed in donkeys exposed to cigarette smoke[15] and after administration of cholinergic drugs.[11] The animals were completely unsedated at all times.

respiratory mechanics

Pulmonary resistance (R_l) and dynamic compliance (C_{dyn}) were determined from simultaneous tracings of pleural pressure, tidal volume, and air flow rate obtained using an Electronics for Medicine VR-6 Pulmonary Function Analyzer.

Pleural pressure was estimated using the esophageal balloon technique.[16] A differential pressure transducer was connected to catheters which extended through the nasal passages, with one terminating in the nasopharynx and the other in an esophageal balloon; nasal resistance was, therefore, not included in measurements of R_l. Flow rate was measured with a screen-type pneumotachograph and pressure transducer; volume was the electrical integration of the flow signal with time. Figure 1 shows a donkey undergoing respiratory function measurements.

Both R_l and C_{dyn} were calculated by the method of Amdur and Mead,[17] the latter by relating volume to change in pressure at points of zero flow at the beginning and end of inspiration, and the former by relating pressure change to change in flow rate at isovolumetric levels of the inspiratory and expiratory phases of a single breath.

During each period within which respiratory function measurements were made, ten technically satisfactory breaths were obtained, and mean values of R_l and C_{dyn} calculated from these were used in all subsequent analyses.

generation of sulfate aerosols

Aerosols of $(NH_4)_2SO_4$ and H_2SO_4 were produced by nebulization of solutions of these materials, using a DeVilbiss no. 40 nebulizer for the latter and a Monaghan no. 670 ultrasonic nebulizer for the former. The output from either nebulizer was mixed with 16 liters/min of heated (110°C), filtered air and then with 75-100 liters/min of filtered dilution air, and passed through two cooling and mixing chambers to breathing and sampling ports; air at the breathing port was maintained at 28°C, 45% relative humidity, and atmospheric pressure. Aerosol output mass concentration was regulated by controlling the concentration of the appropriate sulfate solution in the nebulizer, the output rate of the nebulizer, and the volume of dilution air. Samples of the aerosol for mass

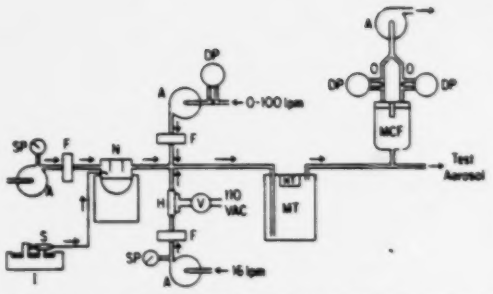

Figure 2 — Schematic diagram of apparatus used to generate $(NH_4)_2SO_4$ and H_2SO_4 aerosols.

A — Air mover
F — Air filter
H — Heater
I — Infusion pump
N — Nebulizer (ultrasonic nebulizer shown here)
O — Orifice type flow meter
S — Syringe
V — Variable voltage transformer
HT — Hygrometer-thermometer unit
MT — Mixing tank
DP — Differential pressure gauge
SP — Static pressure gauge
MCF — Multiple cyclone-filter sampler

concentration and particle size analyses were collected using a pre-calibrated multicyclone sampler[18] containing two cyclones; sulfate content of the cyclones and back-up filters within the sampler was determined using the ASTM turbidometric technique.[19] A Bausch and Lomb spectrophotometer was calibrated with Na_2SO_4 standards for use in these turbidity measurements. Figure 2 shows a schematic diagram of the sulfate aerosol generation unit. Tests showed that both the mass concentration and particle size of the aerosol was the same at the sampling and exposure ports of the generation unit. The output aerosol ranged in size from 0.3 μm to 0.6 μm aerodynamic median diameter (AMD) ($\sigma_g \simeq 1.5$).

aerosol inhalation

The donkeys inhaled both the ferric oxide and sulfate aerosols *ad lib*, through 0.95 cm i.d. nasal catheters which extended into the nasopharynx; this minimized particle deposition in the head. The catheters passed through a flexible film bag, which extended over the muzzle. The top edges of the bag were sealed around the muzzle with tape. Excess aerosol and exhaled particles were collected on a filter canister (Figure 3b). All sulfate aerosol exposures were for one hour; ferric oxide exposures lasted less than five minutes.

experimental protocols

respiratory mechanics

Prior to sulfate exposure, baseline control values for R_l and C_{dyn} were obtained by taking at least three successive sets of ten measurements, to be certain of a stable breathing pattern. This allowed determination of spontaneous variability of individual measurements. The first post-exposure measurements were obtained within one minute of the end of the sulfate aerosol exposure period.

To determine any effect upon mechanics due solely to breathing from the sulfate generation unit, R_l and C_{dyn} were measured following five sham exposures of each donkey, in which the appropriate nebulizer was filled with water, and the animal inhaled from the generation unit for one hour. These sham exposures had no effect upon R_l or C_{dyn} ($p > 0.05$).

regional deposition and clearance

A series of eleven control tests was performed on each animal to determine baseline values for percentage 24-hour clearance, bronchial clearance half-time, and tracheal transport rate. Additional control tests were performed on the day preceding a sulfate exposure, and occasionally on the day following.

To determine the effect of sulfate exposure upon regional deposition, each donkey was

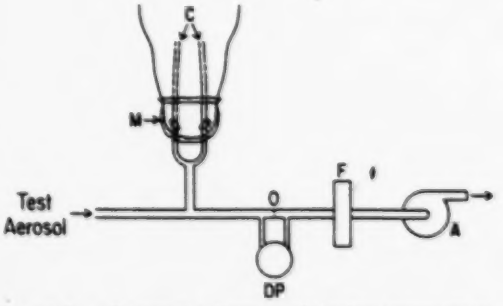

Figure 3 — Schematic diagram of donkey exposure to aerosol.

A — Air mover
C — Nasal catheters
F — Air filter
M — Inhalation mask
O — Orifice type flow meter
DP — Differential pressure gauge

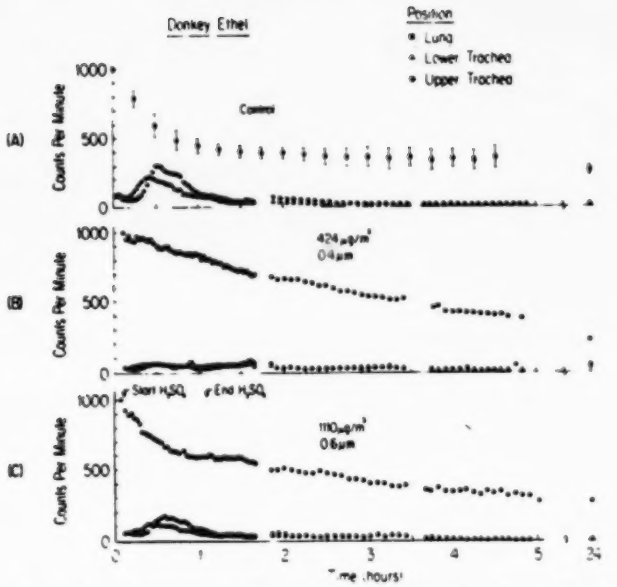

Figure 4 — Clearance curves of radioactively tagged ferric oxide test aerosols for Donkey Ethel. Count rates are normalized to 1000/minute in lung position. (A). Composite control curve. Each point in the lung region represents the mean of 11 tests. Lines through each point represent ± 1 standard deviation. (B). Clearance of ferric oxide inhaled following one-hour exposure to H_2SO_4. (C). Clearance of ferric oxide inhaled immediately preceding H_2SO_4 exposure. Note less slowing of initial clearance compared to (B), and apparent decrease in rate of clearance at ~ 40 min.

exposed to sulfate, followed, within a minute, by inhalation of the tagged ferric oxide aerosol. The ferric oxide retention curves depend upon the pattern of deposition. Therefore, in order to be certain whether an agent affects mucociliary clearance, it must be administered subsequent to inhalation of ferric oxide, so that any effects will be produced solely on clearance without affecting deposition. Thus, exposures were also performed in which ferric oxide was inhaled prior to the one-hour exposure to sulfate.

Three sham exposure controls using each of the above two protocols were performed on each donkey; these had no effect upon regional deposition or clearance ($p > 0.05$).

measurement of regional particle deposition and tracheobronchial mucociliary clearance

Regional deposition and mucociliary clearance patterns were obtained from external, serial measurements of the retention of monodisperse ($\sigma_g < 1.1$), ~5 μm (AMD), insoluble ferric oxide (Fe_2O_3) aerosols tagged with a nonleaching, γ-emitting radioisotope, ^{99m}Tc ($T_{1/2} = 6$ hr.). These aerosols were produced with a spinning disc generator. Measurements were made using an array of three pairs of 12.7 cm (5 in.) diam. x 1.2 cm (0.5 in.) (Tl-activated) scintillation detectors mounted on a saddle worn by the donkey (Figure 1).[20] This detector arrangement allowed measurement of radioactive aerosol in three regions: a major portion of the lung, the lower trachea, and the upper trachea. Clearance from the lung region was seen as a progressive decrease in the measured level of radioactivity (corrected for natural decay) due to the proximal mucociliary transport of deposited particles within the tracheobronchial tree (Figure 4); subsequent clearance waves occurred in the lower and then the upper tracheal regions.

Clearance from the tracheobronchial tree is essentially complete within 24 hrs. following

TABLE I
Respiratory Mechanics

Donkey		Mass Concentration ($\mu g/m^3$)	Particle Size (μm)	R_L Mean Control[a] ($cmH_2O/L/sec$)	R_L Change After Exposure[b] (% of Mean Control)	R_L Minimal Significant Change[c] (% of Mean Control)	C_{dyn} Mean Control[a] (L/cmH_2O)	C_{dyn} Change After Exposure[b] (% of Mean Control)	C_{dyn} Minimal Significant Change[c] (% of Mean Control)
Ethel	H_2SO_4	1405	0.4	2.20	+10.8	26.7	0.28	- 2.1	5.9
	$(NH_4)_2SO_4$	2134	0.4	1.65	- 12.1	33.3	0.34	- 2.3	9.2
Gus	H_2SO_4	1506	0.4	1.24	- 18.5	31.6	0.52	- 4.8	14.3
	$(NH_4)_2SO_4$	2095	0.4	1.10	+30.9	32.1	0.40	+ 6.8	15.3
Harold	H_2SO_4	1440	0.4	2.11	+19.9	40.9	0.26	- 7.5	10.4
	$(NH_4)_2SO_4$	2103	0.4	1.66	- 11.3	26.5	0.33	+ 3.5	11.9
Jack	H_2SO_4	1333	0.4	1.88	+ 4.3	29.2	0.25	+ 2.8	9.3
	$(NH_4)_2SO_4$	2101	0.4	1.80	+ 2.2	28.3	0.33	- 11.4	23.5

[a]Represents the mean of the set of ten control measurements performed immediately prior to sulfate aerosol exposure.
[b]Represents the percentage change of the mean for ten breaths immediately following sulfate exposure from the control mean.
[c]Represents the minimal change for significance at $p = 0.05$.[24]

exposure; residual chest activity remaining at this time represents that portion of the inhaled aerosol which deposited within the nonciliated, alveolar region.[8,21] Each animal has a characteristic and reproducible pattern of regional deposition and clearance for the 5 μm ferric oxide aerosol,[8] and changes in this pattern following H_2SO_4 or $(NH_4)_2SO_4$ exposure would indicate that these latter aerosols were responsible for the alterations.

All clearance tests were analyzed in terms of a characteristic index, the bronchial clearance half-time ($T_{(50\%)}$), *i.e.*, the time required for completion of one-half of the total tracheobronchial clearance of the ferric oxide.[10,11] This parameter has been shown to be sensitive to clearance alterations produced by toxicants and drugs.[22] The index of regional deposition alteration was change in the percentage of the initial lung burden cleared by 24-hrs. The rate of mucociliary transport in the trachea was also determined, in terms of the time displacement of the activity waves in successive measurement positions, based upon the points of one half-maximum count rate of the ascending portion of each wave. The actual transport rate was then obtained by dividing the distance between tracheal measurement positions by this time interval displacement.[11]

The donkeys were housed in an unheated shed during the course of this study. Because of the effect of ambient pretest temperature upon mucociliary transport rates and fractional deposition in the tracheobronchial tree, values for $T_{(50\%)}$, 24-hr. clearance, and tracheal transport rate were corrected to room temperature (20°C), using the correction factors determined in this Laboratory by Bohning, *et al.*[23]

The donkeys had been exposed to sulfate aerosols prior to the initiation of the deposition and clearance studies, in the series of tests performed to determine the effects on respiratory mechanics. There is, however, no indication that these latter studies produced any effects which persisted through the period of at least two months which elapsed between the last of the mechanics exposures, and the start of those for deposition and clearance tests. Furthermore, two of the animals (*i.e.*, Donkeys Harold and Gus) had been used in previous experimental studies and the percentage 24-hr. clearance, $T_{(50\%)}$ and tracheal transport rates values of control tests in these previous studies were not significantly different ($p > 0.05$) from those obtained in the initial eleven control tests in the current study.

results

respiratory mechanics

Each donkey was exposed to mass concentrations of $(NH_4)_2SO_4 > 400\ \mu g/m^3$, and to $H_2SO_4 > 800\ \mu g/m^3$ for one hour. Table I shows results of tests at the highest levels used. None of the changes in mechanics immediately following

TABLE II
Regional Deposition of Tagged Ferric Oxide Test Aerosol

	Mean Control		H_2SO_4 Exposure			$(NH_4)_2SO_4$ Exposure		
Donkey	Tracheobronchial Deposition[a] (% of Initial Lung Burden Cleared by 24-Hrs)	Minimal Significant Change[b] (% of Mean Control)	Mass Concentration ($\mu g/m^3$)	Particle Size (μm)	Change in Tracheobronchial Deposition Following Exposure (% of Mean Control)	Mass Concentration ($\mu g/m^3$)	Particle Size (μm)	Change in Tracheobronchial Deposition Following Exposure (% of Mean Control)
Ethel	72 (6)[c]	10.8	71	0.3	-9.7			
			444	0.4	+9.7	290	0.3	-1.4
			1364	0.4	-8.6	2643	0.6	0
Gus	71 (6)	10.2	136	0.4	-9.5			
			894	0.4	-5.6	520	0.3	+4.2
			1220	0.6	+8.2	2785	0.4	0
Harold	68 (5)	6.8	85	0.3	+2.9	600	0.3	+5.3
			331	0.5	0	3140	0.5	+5.1
			993	0.5	+4.4			
Jack	75 (7)	9.2	78	0.3	+8.3	490	0.3	-1.3
			351	0.3	-5.3	1985	0.3	+2.6
			1032	0.4	-6.7			

[a] Based upon eleven tests performed prior to the sulfate aerosol exposure series.
[b] Represents the minimal change for significance at $p = 0.05$.[24]
[c] Standard deviation.

sulfate exposure were significantly different from the pre-exposure controls ($p > 0.05$).

Control tests of respiratory mechanics showed there to be a fairly large, random intra-animal variability in repeated measurements of R_1, and to a lesser extent of C_{dyn}, within each series of ten breaths. This variability resulted in the necessity for relatively large changes in R_1 and C_{dyn} to occur in order for the postexposure values to be significantly different from pre-exposure controls. Table I shows the minimal percentage change in the mean of the last set of pre-exposure measurements which was necessary in order to lead to a conclusion of significant effect due to sulfate exposure (Type I error probability = 0.05, Type II error probability = 0.50).[24] The greater variability of R_1 is reflected in larger changes needed for significance than required for C_{dyn}; in addition, some animals were more variable than others. All changes in R_1 and C_{dyn} following sulfate exposures were generally well below these minimal significant measurement limits.

Exposures to lower concentrations of $(NH_4)_2SO_4$ and H_2SO_4 than those shown in Table I also produced no significant changes in R_1 or C_{dyn}; similar minimal changes for significance were necessary in these tests.

regional deposition

Each donkey was exposed to various concentrations of $(NH_4)_2SO_4$ and H_2SO_4 for one hour to assess any effect upon the regional deposition pattern of the radioactive ferric oxide test aerosol subsequently inhaled. Table II shows the results of selected tests, including the lowest and highest concentration used for each animal. No significant change ($p > 0.05$) in the percentage of the radioactive ferric oxide test aerosol cleared by 24-hrs. was observed in any of these tests, nor for any of the other intermediate level exposures performed.

tracheobronchial mucociliary clearance

Because regional deposition was not affected by sulfate exposure, protocols in which sulfate aerosol was inhaled either prior to or following ferric oxide inhalation were both used to assess any effect upon clearance; some of these tests had originally been performed to determine the effect of sulfate upon deposition.

In order to compare individual values for $T_{(50\%)}$ of the ferric oxide test aerosol obtained following each sulfate exposure, upper tolerance limits for the normal $T_{(50\%)}$ were determined for each donkey, based upon the eleven control tests performed prior to the start of the sulfate exposure series. Each limit was chosen so that there would be a 95% probability that at least 99% of the theoretical population of calculated normal $T_{(50\%)}$ values would lie below the

TABLE III
Tracheobronchial Clearance of Tagged Ferric Oxide Test Aerosol

Donkey	Mean Control $T_{(50\%)}$* (Min)	$T_{(50\%)}$ Upper Tolerance Limit* (Min)	$(NH_4)_2SO_4$ Exposure† Mass Concentration ($\mu g/m^3$)	Particle Size (μm)	$T_{(50\%)}$ Following Exposure (Min)
Ethel	23 (4)‡	40	290	0.3	34
			2643	0.6	30
Gus	22 (3)	35	520	0.3	34
			2785	0.4	21
Harold	26 (2)	34	600	0.3	28
			3140	0.5	30
Jack	27 (3)	39	490	0.3	32
			1985	0.3	34

*Based upon eleven tests performed prior to sulfate aerosol exposure series.
†$(NH_4)_2SO_4$ was inhaled prior to Fe_2O_3 inhalation.
‡Standard deviation.

limits.[25] A significant difference from controls ($p<0.05$) is, therefore, assigned to any test value of $T_{(50\%)}$ lying above these limits. The upper tolerance limits for each donkey are presented in Table III, along with the mean values for $T_{(50\%)}$ of the eleven control tests.

The values for $T_{(50\%)}$ obtained following ammonium sulfate exposure are also shown in Table III. No significant change was observed in $T_{(50\%)}$ for any of the animals at any of the concentrations used.

Figure 5a-d shows values for $T_{(50\%)}$ obtained following one hour exposures to H_2SO_4. These acid exposures provided evidence for a large, inter-animal difference in sensitivity, as well as evidence for two types of response. Three of the animals showed pronounced immediate slowing of clearance following some of the acid exposures. One of these three and the fourth animal showed a significant, progressive change in the control clearance half-time over the course of the series of exposures to acid mist.

Donkeys Ethel and Gus exhibited increased $T_{(50\%)}$ values following single exposures to H_2SO_4 without showing any progressive changes in control test $T_{(50\%)}$ values. Ethel is, however, the more sensitive of the pair, showing significantly lengthened $T_{(50\%)}$ at concentrations of acid mist as low as 194 $\mu g/m^3$, and at all concentrations $\geq$ 314 $\mu g/m^3$ when acid was inhaled prior to ferric oxide inhalation. On the other hand, Donkey Gus appears to be more resistant to the acid, showing a significantly lengthened $T_{(50\%)}$ only at concentrations $\geq$ 694 μ/m^3.

A composite control clearance curve is shown in Figure 4a for Donkey Ethel. Figure 4b shows the clearance pattern obtained for radioactive ferric oxide aerosol inhaled following acid exposure, while Figure 4c shows clearance of the ferric oxide aerosol inhaled prior to acid exposure. The maximum effect upon clearance was produced when the acid mist was inhaled prior to the ferric oxide. In the reverse protocol, clearance appears to proceed normally during at least the first half-hour of acid exposure, followed by an apparent, transient slowdown between 30 and 60 minutes. This suggests that the acid requires at least 30 minutes to produce an effect, during which time a large fraction of the total tracheobronchial clearance is completed.

Except for the control test performed the day following acid exposure at 424 $\mu g/m^3$, all controls for Donkey Ethel were below the tolerance limit. Donkey Gus exhibited two control values outside his limit: on the day following exposure to 694 $\mu g/m^3$ acid, and a control performed the next week.

Donkeys Harold and Jack exhibited progressive alterations in their control clearance half-times during the period of weekly exposures to acid mist. The observed change in Harold was much greater than that in Jack, even though the former was involved in fewer tests at lower concentrations, both prior to and during the period of elevated controls.

Unlike Donkey Harold, Donkey Jack showed no transient lengthening of $T_{(50\%)}$ following

TABLE IV
Tracheal Transport Rate of Tagged Ferric Oxide Test Aerosol

Donkey	Mean Control Tracheal Transport Rate[a] (cm/min)	Minimal Significant Change[b] (% of Mean Control)	Test Run	Change in Tracheal Transport Rate (% of Mean Control)
Gus	1.2 (0.3)[c]	15.2	Following H_2SO_4 exposure, 989 $\mu g/m^3$	- 8.3
Harold	1.9 (0.3)	17.3	Control, with lengthened $T_{(50\%)}$	+10.5
			Following H_2SO_4 exposure, 331 $\mu g/m^3$	+ 5.3
Jack	1.3 (0.2)	17.7	Control, with lengthened $T_{(50\%)}$	+ 7.6
			Following H_2SO_4 exposure, 596 $\mu g/m^3$	- 10.8

[a] Based upon eleven tests performed prior to sulfate aerosol exposure series.
[b] Represents the minimal change for significance at $p = 0.05$.[24]
[c] Standard deviation.

single acid exposures. However, Jack does show an apparent speeding-up of clearance (shorter $T_{(50\%)}$ in the three acid exposures (at 596, 246 and 278 $\mu g/m^3$) performed where the pre-exposure control was significantly slower than normal. No speed-up, however, was observed compared to normal control values of $T_{(50\%)}$.

tracheal transport rate

Tracheal transport rate could only be determined in tests with clearly discernable tracheal peaks; severe depression of lung clearance was often associated with an absence of such peaks (Figure 4b).

Table IV presents tracheal transport rate data for selected tests in which H_2SO_4 exposure resulted in slowed bronchial clearance. None of the changes in tracheal transport rate were significantly different ($p > 0.05$) from the control rates for these, or any of the other tests. Data are also included for selected control tests of Donkeys Harold and Jack in which $T_{(50\%)}$ was altered by repeated acid exposures. There were no significant changes in tracheal transport rate in these tests.

discussion

Changes in bronchial clearance rate were observed in this study at H_2SO_4 concentrations which produced no measurable effect upon regional deposition, pulmonary resistance, or dynamic compliance, indicating that alteration in clearance rate may be a sensitive indicator of acute response to submicron respiratory irritants.

Any change in pulmonary resistance due to aerosol induced irritant bronchoconstriction is dependent upon the sites of initial particle deposition, the concentration of irritant receptors in these areas, and the relative contribution of any bronchoconstriction in these areas to the total measured resistance. Studies of normal human and animal lungs[26,27] have shown that the resistance of small airways ($< \sim 2$ mm diam.) accounts for only a small fraction of the total resistance of the tracheobronchial tree. Selective constriction in these airways could, therefore, have little effect upon measured total R_l; thus, change in R_l may only be a sensitive index of irritant response in the larger ($\geq \sim 2$ mm diam.) airways.

It is likely that the H_2SO_4 and $(NH_4)_2SO_4$ aerosols deposited largely in the small airways. The median sizes of the test aerosols were 0.3-0.6 μm. However, in the humid atmosphere of the respiratory tract, these particles would increase in size fairly rapidly. The exact growth in the respiratory tract was not determined, although at the very high relative humidity therein, growth may theoretically continue indefinitely, with the final size depending upon the residence time in the tract.[28] Recent work in this laboratory[29] indicates growth to about twice the original size. The lack of measurable effect of H_2SO_4 upon tracheal transport rate in tests in which bronchial clearance was altered also suggests that the site of action of the acid mist was deeper within the lung.

Changes in small airway calibre, if not reflected in altered R_l, may be detected by an alteration in the pattern of regional particle deposition. Bronchoconstriction has been shown to affect particle deposition patterns in the lung. In a pilot test series performed in this

laboratory,[30] exposures of two people to 12-13 ppm SO_2 for 6-7 minutes produced both a marked decrease in the alveolar zone deposition of 5-6 μm Fe_2O_3 particles and a significant change in R_1; in two other exposures at 5 and 9 ppm, no shift in regional deposition nor change in R_1 was seen. Fairchild, *et al.*[31] found a proximal shift towards the upper respiratory tract of radiolabelled Streptococcus aerosol in guinea-pigs exposed for one hour to concentrations of H_2SO_4 mist known to cause nasal airway constriction. Cigarette smoke is associated with increases in R_1,[32] and with altered patterns of regional deposition.[33] In addition, good correlations have been found between increased nasal resistance and increased nasopharyngeal particle deposition within individual human subjects.[34] Thus, the lack of observed effect upon regional deposition in the donkeys is consistent with a lack of observed effect upon R_1.

The inter-animal differences in response sensitivity of the mucociliary system to H_2SO_4 mist, even in this small group of four animals, is not surprising. Individual humans differ widely in their response to inhaled irritants; exposures that severly affect one may have no effect upon another.[35] Although the reasons for this are unclear, they probably involve variability in physiological and anatomical mechanisms, *e.g.* responsiveness of irritant receptor cells. Wide ranges of sensitivity have been shown with respect to changes in R_1 due to SO_2 exposure in healthy humans.[36] Interindividual variability in other biological responses to inhaled irritants, such as change in mucociliary clearance, may be expected.

Examination of Figure 5a-d shows that repeated acid exposures in an individual animal at comparable concentrations and particle size did not produce comparable changes in $T_{(50\%)}$. Furthermore, response to H_2SO_4 appeared to be erratic, with no clear dose-response pattern of progressively lengthening $T_{(50\%)}$ with increasing mass concentration of H_2SO_4. A possible explanation for these observations is provided by Larson *et al.*[37] Gaseous ammonia has been found to be a component of expired air in man[38] with amounts variable within and among individuals; preliminary work in this laboratory has indicated that ammonia is a component of the expired air in the donkey.[39] Larson *et al.* Propose that the ammonia in the respiratory tract neutralizes inhaled, airborne acid aerosols, altering or mitigating their toxicity by reducing acidity before deposition occurs, via conversion to $(NH_4)_2SO_4$; the current study has shown that $(NH_4)_2SO_4$ has no measurable effect upon clearance. Thus, variability in the degree of neutralization on different test days could result in different effective concentrations of acid depositing in the tracheobronchial tree within each individual animal, resulting in variable degrees of response to virtually identical acid exposures.

The progressive change in normal clearance pattern of the two healthy animals observed during the course of weekly one-hour exposures to H_2SO_4 mist is a very significant observation. The study of Sim and Pattle[40] on healthy human males suggests the development of chronic respiratory effects due to repeated exposures to H_2SO_4 mist. Two subjects developed what appeared to be long-lasting bronchitic symptoms due to repeated exposures of $\leqslant$ 60 min. each, given no more than twice a week with at least 24 hrs. between exposures.

Any persistent alteration of normal mucociliary clearance can have important pathological implications. The mucociliary system is a prime defense against harmful airborne pollutants, acting to transport inhaled deposited particles, pathogenic organisms and absorbed gases (all of which often coexist with sulfur oxides in community and occupational environments) out of the respiratory tract. An inefficient system may result in prolonged retention, rendering the host more susceptible to injury and/or disease.

Other studies have shown tracheobronchial clearance rate to be affected by various respiratory irritants. Alterations in clearance have been observed following acute exposures to SO_2 ($>$ 300 ppm for 1/2 hr.) in donkeys,[9] cigarette smoke in both donkeys and humans,[10-13] and H_2SO_4 mist (15 mg/m^3, 3.2 μm CMD for 4 hrs.) in mice.[41] Retardation of clearance due to exposure to respiratory irritants could possibly be a factor in exacerbations of morbidity during acute air pollution episodes.

The mass concentrations of H_2SO_4 which produced clearance alterations in this study are

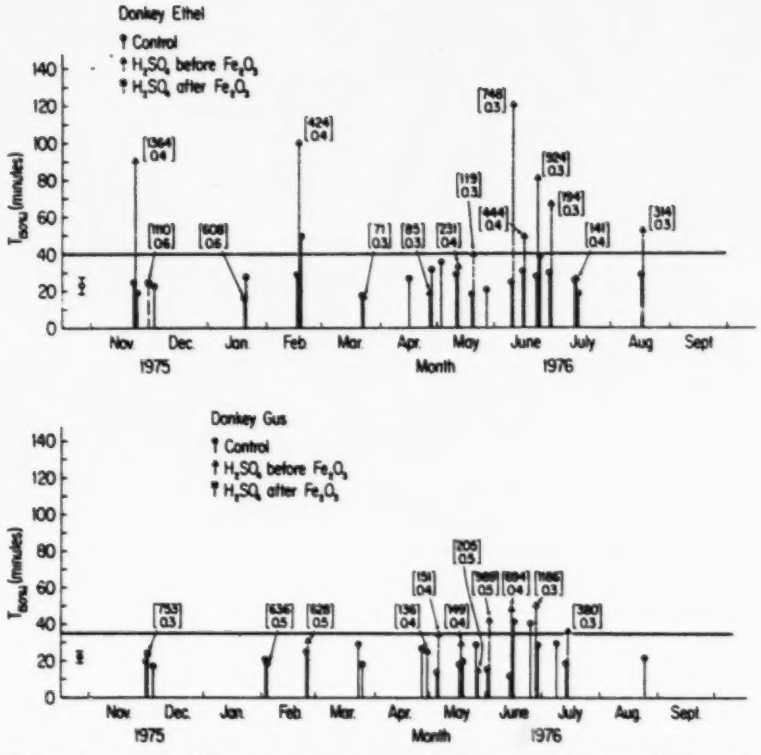

Figure 5 — Bronchial clearance half-times following exposures to H_2SO_4, with controls. The mean (± standard deviation) $T_{(50\%)}$ of the eleven control tests performed

above the average levels currently found in ambient air; most were, however, well below the occupational 8-hour threshold limit value (1000 $\mu g/m^3$).[42] In addition, community levels may be increasing due to the emission of H_2SO_4 mist from automobile catalytic converters and the increased conversion of SO_2 injected into the atmosphere by tall stacks of electric power plants. Furthermore, intermittent community exposures may extend for periods much greater than one hour.

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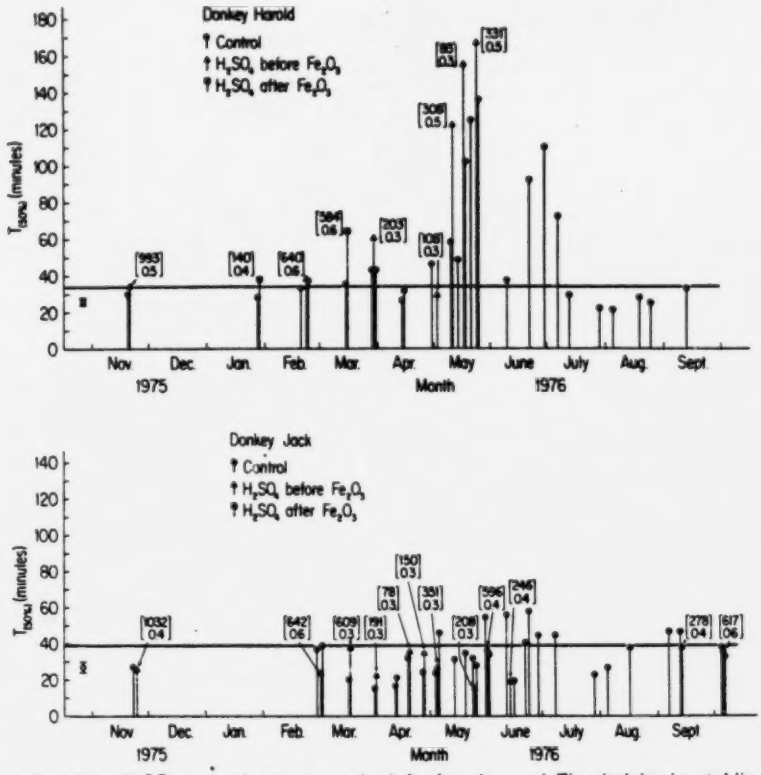

prior to the H_2SO_4 series is shown at the left of each panel. The dark horizontal line represents the upper tolerance limit for $T_{(50\%)}$ based upon these controls.

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